

Use of Novel Cytokine Receptors as Biomarkers and Therapeutic Targets in Human Cancer

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Field of the Invention

10 The present invention concerns nucleic acids encoding erythropoietin receptor isoforms, proteins encoded by such nucleic acids, antibodies that bind to such proteins, and methods of using the same.

Background of the Invention

15 Erythropoietin (Epo) is the principal hematopoietic growth factor that promotes the viability, differentiation and proliferation of mammalian erythroid progenitor cells (S. Krantz, *Blood* **77**, 419-34 (1991)). The biologic effects of Epo are mediated via its interaction with its specific transmembrane receptor, EpoR (H. Youssoufian, *Blood* **81**, 2223-36 (1993)). The EpoR lacks intrinsic tyrosine kinase
20 activity and upon ligand binding activates a receptor-associated tyrosine kinase Jak2 which is critical for anti-apoptosis and mitogenic signaling via the EpoR (O. Miura et al., *Blood* **84**, 1501-7 (1994); B. Witthuhn et al., *Cell* **74**, 227-36 (1993); J. Ihle, *Nature* **337**, 591-4 (1995); H. Zhuang et al., *J. Biol. Chem.* **270**, 14500-4 (1995)). Activated Jak2 then phosphorylates a number of cytoplasmic proteins as well as the
25 EpoR itself. Expression of Epo receptors has been reported on several non-hematopoietic cell types including vascular endothelial cells, placental tissue, neuronal cells, kidney and cardiomyocytes (A. Anagnostou et al., *Proc. Natl. Acad. Sci. USA* **91**, 3974-8 (1994); S. Masuda et al., *J. Biol. Chem.* **268**, 112-8-16 (1993); S. Sawyer et al., *Blood* **74**, 103-9 (1989); M. Wald et al., *J. Ce.. Physiol.* **167**, 461-8
30 (1996)).

 Recombinant human Epo (r-HuEpo) has been widely used in many different types of cancers for the treatment or prevention of chemo-radiotherapy induced anemia (A. Moliterno and J. Spivak, *Hematol. Oncol. Clin. North Am.* **10**, 345-63 (1996)). For instance, in patients with breast cancer, r-HuEpo has been investigated

in clinical trials for its potential beneficial effects in the prevention or treatment of chemotherapy or radiation therapy-related anemia (L. Del Mastro et al., *J. Clin. Oncol.* **15**, 2715-21 (1997); H. Ludwig et al., *Ann. Oncol.* **4**, 161-7 (1993); P. Sweeney et al., *Br. J. Cancer* **77**, 1996-2002 (1998); S. Vijayakumar et al., *Int. J. Radiat. Oncol. Biol. Phys* **26**, 721-9 (1993)), for mobilization of peripheral blood progenitor cells (C. Waller et al., *Bone Marrow Transplant* **24**, 19-24 (1999)), to increase the rate of hematopoietic recovery following high dose chemotherapy (P. Benedetti Panici et al., *Br. J. Cancer* **75**, 1205-12 (1997); S. Filip et al., *Neoplasma* **46**, 166-72 (1999)) as well as use in ex vivo expansion strategies of stem cells (C. Bachier et al., *Exp Hematol.* **27**, 615-23 (1999); L. Pierelli et al., *Exp. Hematol.* **27**, 416-24 (1999); P. Stiff et al., *Blood* **95**, 2169-74 (2000); W. Vogel et al., *Blood* **86**, 1362-7 (1996)). Similarly, r-HuEpo has been investigated in several clinical trials of squamous cell cancers of head-neck (F. Dunphy et al., *Cancer* **86**, 1362-7 (1999); M. Henke et al., *radiother Oncol* **50**, 185-90 (1999); G. Mantovani et al., *Oncol. Rep.* **6**, 421-6 (1999)) and uterine cervix (K. Dusenbery et al., *Int. J. Radiat. Oncol. Biol. Phys.* **29**, 1079-84 (1994)).

In view of the foregoing, it would be extremely desirable to understand the association of Epo with tumor growth and how EpoR may be involved in cancer pathophysiology and progression.

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Summary of the Invention

A first aspect of the present invention is an isolated nucleic acid encoding erythropoietin isoform 1, erythropoietin isoform 2, erythropoietin isoform 3, erythropoietin isoform 4, or erythropoietin isoform 5, or a nucleic acid that encodes the opposite or complementary strand of a nucleic acid as set forth above (e.g., a DNA encoding an RNA).

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A second aspect of the present invention is a protein encoded by a nucleic acid as described above (e.g., an isolated and/or purified protein).

A third aspect of the present invention is an antibody that selectively or specifically binds to a protein as described above.

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A further aspect of the present invention is an oligonucleotide probe that selectively or specifically binds to a nucleic acid as described above.

A further aspect of the present invention is a method of screening a subject for cancer, comprising: detecting the presence or absence of a nucleic acid encoding an

isoform as described above in the subject, the presence of such a nucleic acid indicating the subject is afflicted with or at risk of developing cancer.

A further aspect of the present invention is a method of screening a subject for cancer, comprising detecting the presence or absence of a protein or isoform as described above in the subject, the presence of such a protein indicating the subject is afflicted with or at risk of developing cancer.

Particular cancers which may be screened by the methods described herein include, but are not limited to, breast, cervix, ovarian, prostate, colon and lung cancer.

The foregoing and other objects and aspects of the present invention are explained in detail in the drawings herein and the specification set forth below.

Brief Description of the Drawings

Figure 1. The organization of the EpoR gene (GenBank accession number S45332, **SEQ ID NO: 1**). The splicing that results in the mature mRNA for the wild-type receptor (**SEQ ID NO: 3**), and five alternatively spliced isoforms (1-5) described herein are depicted schematically. The translated regions of the gene are indicated in black, whereas untranslated regions are indicated in white. Novel amino acid translations that result from alternative splicing of the EpoR gene transcript are indicated in grey.

Figure 2. Changes in the open reading frames (ORFs) of mature mRNA sequences from the full-length wild-type receptor in the isoforms of EpoR described herein. **Isoform 1 (SEQ ID NO: 4):** Additional nucleotides from intron 6 (nucleotides 5949-6062, **SEQ ID NO: 1**) are spliced between exons 6 and 7. **Isoform 2 (SEQ ID NO: 6):** Splicing at the 5' end of exon 8 occurs 19 nucleotides upstream (nucleotide 7498) from that seen in the full-length wild-type message (nucleotide 7517). **Isoform 3 (SEQ ID NO: 8):** Intron 7 is not spliced out of the final message. **Isoform 4 (SEQ ID NO: 10):** Intron 5 is not spliced out of the final message. **Isoform 5 (SEQ ID NO: 12):** exon 6 is skipped, with exon 5 spliced directly to exon 7. Putative C-terminal amino acid sequence changes from wild-type EpoR are depicted in **bold**.

Detailed Description of the Preferred Embodiments

The present invention is explained in greater detail below. This description is not intended to be a detailed catalog of all the different ways in which the invention

may be implemented, or all the features that may be added to the instant invention. For example, features illustrated with respect to one embodiment may be incorporated into other embodiments, and features illustrated with respect to a particular embodiment may be deleted from that embodiment. In addition, numerous variations and additions to the various embodiments suggested herein will be apparent to those skilled in the art in light of the instant disclosure which do not depart from the instant invention. Hence, the following specification is intended to illustrate some particular embodiments of the invention, and not to exhaustively specify all permutations, combinations and variations thereof.

Nucleic acid as used herein refers to any type of nucleic acid, including naturally occurring and synthetic nucleic acids and including both DNA and RNA.

Subjects with which the present invention may be carried out are generally mammalian subjects, including both human subjects and non-human subjects (*e.g.*, dog, cat, horse, rabbit, rat) for veterinary or research purposes.

Any type of antibody may be used in the present invention. The term "antibodies" as used herein refers to all types of immunoglobulins, including IgG, IgM, IgA, IgD, and IgE. Of these, IgM and IgG are particularly preferred. The antibodies may be monoclonal or polyclonal (with monoclonal antibodies preferred) and may be of any species of origin, including (for example) mouse, rat, rabbit, horse, or human. See, *e.g.*, M. Walker et al., *Molec. Immunol.* **26**, 403-11 (1989). Antibody fragments that retain specific binding to the protein or epitope bound by the antibody are included within the scope of the term "antibody" and include, for example, Fab, F(ab')₂, and Fc fragments, and the corresponding fragments obtained from antibodies other than IgG. Such fragments can be produced by known techniques. The antibodies may be chimeric or humanized, particularly when they are used for therapeutic purposes.

Applicants specifically intend that all United States patent references cited herein be incorporated herein by reference in their entirety.

1. Nucleic acids.

As noted above, a first aspect of the present invention is a nucleic acid encoding an erythropoietin receptor isoform as described herein. In certain embodiments the nucleic acid may be an RNA such as an mRNA, or may be a DNA.

In one embodiment, the nucleic acid encodes erythropoietin receptor isoform 1 and has the sequence given herein as **SEQ ID NO: 4**.

In another embodiment, the nucleic acid encodes erythropoietin receptor isoform 2 and has the sequence given herein as **SEQ ID NO: 6**.

5 In another embodiment, the nucleic acid encodes erythropoietin receptor isoform 3 and has the sequence given herein as **SEQ ID NO: 8**.

In another embodiment, the nucleic acid encodes erythropoietin receptor isoform 4 and has the sequence given herein as **SEQ ID NO: 10**.

10 In another embodiment, the nucleic acid encodes erythropoietin receptor isoform 5 and has the sequence given herein as **SEQ ID NO: 12**.

In another embodiment, the nucleic acid that encodes the opposite strand of a nucleic acid as set forth above (*e.g.*, is a DNA encoding an RNA).

Nucleic acids as described above may be natural or synthetic, and can be produced in accordance with techniques known in the art or variations thereof which
15 will be apparent in light of the disclosure herein.

Nucleic acids as described above may be coupled to appropriate regulatory elements such as a promoter to produce a recombinant nucleic acid construct, which construct may be inserted into a host cell in which the promoter is operable so that the encoded protein is expressed by the host cell. Recombinant techniques and the
20 production of proteins in recombinant cells may be carried out in accordance with known techniques.

2. Antibodies.

Polyclonal antibodies used to carry out the present invention may be produced
25 by immunizing a suitable animal (*e.g.*, rabbit, goat, etc.) with the antigen to which the monoclonal antibody binds, collecting immune serum from the animal, and separating the polyclonal antibodies from the immune serum, in accordance with known procedures. Depending on the host species, various adjuvants may be used to increase immunological response. Such adjuvants include, but are not limited to, Freund's,
30 mineral gels such as aluminum hydroxide, and surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanin, and dinitrophenol. Among adjuvants used in humans, BCG (*bacilli Calmette-Guerin*) and *Corynebacterium parvum* are especially preferable.

Monoclonal antibodies of the present invention may be prepared using any technique which provides for the production of antibody molecules by continuous cell lines in culture. These include, but are not limited to, the hybridoma technique, the human B-cell hybridoma technique, and the EBV-hybridoma technique (Kohler, G. et al. (1975) *Nature* **256**:495-497; Kozbor, D. et al. (1985) *J. Immunol. Methods* **81**:31-42; Cote, R. J. et al. (1983) *Proc. Natl. Acad. Sci.* **80**:2026-2030; Cole, S. P. et al. (1984) *Mol. Cell Biol.* **62**:109-120). Briefly, the procedure is as follows: an animal is immunized with antigen or immunogenic fragments or conjugates thereof. For example, haptenic oligopeptides of antigen can be conjugated to a carrier protein to be used as an immunogen. Lymphoid cells (e.g. splenic lymphocytes) are then obtained from the immunized animal and fused with immortalizing cells (e.g. myeloma or heteromyeloma) to produce hybrid cells. The hybrid cells are screened to identify those which produce the desired antibody.

Human hybridomas which secrete human antibody can be produced by the Kohler and Milstein technique. Although human antibodies are especially preferred for treatment of human, in general, the generation of stable human-human hybridomas for long-term production of human monoclonal antibody can be difficult. Hybridoma production in rodents, especially mouse, is a very well established procedure and thus, stable murine hybridomas provide an unlimited source of antibody of select characteristics. As an alternative to human antibodies, the mouse antibodies can be converted to chimeric murine/human antibodies by genetic engineering techniques. See V. T. Oi et al., *Bio Techniques* **4**(4):214-221 (1986); L. K. Sun et al., *Hybridoma* **5** (1986).

In addition, techniques developed for the production of "chimeric antibodies", the splicing of mouse antibody genes to human antibody genes to obtain a molecule with appropriate antigen specificity and biological activity can be used (S. L. Morrison, et al. *Proc. Natl. Acad. Sci.* **81**, 6851-6855 (1984); M. S. Neuberger et al., *Nature* **312**:604-608 (1984); S. Takeda, S. et al., *Nature* **314**:452-454 (1985)). Alternatively, techniques described for the production of single chain antibodies may be adapted, using methods known in the art, to produce isoform-specific single chain antibodies. Antibodies with related specificity, but of distinct idiotypic composition, may be generated by chain shuffling from random combinatorial immunoglobulin libraries (D. R. Burton, *Proc. Natl. Acad. Sci.* **88**,11120-3 (1991)).

Antibodies may also be produced by inducing *in vivo* production in the lymphocyte population or by screening immunoglobulin libraries or panels of highly specific binding reagents as disclosed in the literature (R. Orlandi et al., *Proc. Natl. Acad. Sci.* **86**, 3833-3837 (1989)); G. Winter et al., *Nature* **349**, 293-299 (1991)).

5 Antibodies that selectively bind to a particular erythropoietin receptor isoform as described herein (*i.e.*, that selectively bind to one of isoforms 1-5 but do not bind to the other of isoforms 1-5) can be identified in accordance with known techniques, such as their ability to compete with labeled antibody to in binding to that isoform in a competitive binding assay.

10 If desired, antibodies specific for a particular isoform can be used to produce anti-idiotypic (paratope-specific) antibodies. *See e.g.*, McNamara et al., *Science* **220**,1325-26 (1984), R. C. Kennedy, et al., *Science* **232**,220 (1986).

3. Immunoassay techniques.

15 Those skilled in the art will be familiar with numerous specific immunoassay formats and variations thereof which may be useful for carrying out the method disclosed herein. See generally E. Maggio, *Enzyme-Immunoassay*, (1980)(CRC Press, Inc., Boca Raton, FL); see also U.S. Patent No. 4,727,022 to Skold et al. titled "Methods for Modulating Ligand-Receptor Interactions and their Application," U.S.
20 Patent No. 4,659,678 to Forrest et al. titled "Immunoassay of Antigens," U.S. Patent No. 4,376,110 to David et al., titled "Immunometric Assays Using Monoclonal Antibodies," U.S. Patent No. 4,275,149 to Litman et al., titled "Macromolecular Environment Control in Specific Receptor Assays," U.S. Patent No. 4,233,402 to Maggio et al., titled "Reagents and Method Employing Channeling," and U.S. Patent
25 No. 4,230,767 to Boguslaski et al., titled "Heterogenous Specific Binding Assay Employing a Coenzyme as Label."

Antibodies as described herein may be coupled or conjugated to a solid support suitable for a diagnostic assay (e.g., beads, plates, slides or wells formed from materials such as latex or polystyrene) in accordance with known techniques, such as
30 precipitation. Antibodies as described herein may likewise be coupled or conjugated to detectable groups such as radiolabels (e.g., ³⁵S, ¹²⁵I, ¹³¹I), enzyme labels (e.g., horseradish peroxidase, alkaline phosphatase), fluorescent labels (e.g., fluorescein), chemiluminescent labels (e.g., acridinium groups, metalloporphyrins such as phthalocyanine dyes, luminol, etc.), metal atoms (e.g., technetium-99m), etc., in

accordance with known techniques. *See, e.g.*, U.S. Patent No. 4,472,509 to Gansow (metal chelates to monoclonal antibodies); U.S. Patent No. 5,061,641 to Schochat et al.; and U.S. Patent No. 4,861,869 to Nicoleotti et al. (radiolabelling proteins).

Immunoassays, or other types of assays to detect and/or quantitate the level of the isoform in samples as described below, may be used in screening assays to detect pathologic states associated with aberrant levels of isoform expression (e.g., tumors, inflammatory states), diagnostic studies, prognostic studies, or to monitor the progression or diminution of isoform expression in correlation with disease state.

Samples that may be collected for use in carrying out the immunoassay may be tissue samples from the organ or tissue of interest within the subject, such tissue generally of most interest being those types of tissues/cells that express differing amounts of isoform in pathologic states as compared to non-pathologic states, or biological fluids such as blood (including blood fractions such as blood plasma or blood serum), urine, cerebrospinal fluid, etc). Examples may include overexpression or aberrant expression of the isoform in various types of malignancies (e.g ovarian cancer, endometrial cancer, pancreatic cancer, breast cancer, urinary bladder cancer, lung cancer, etc.), as well as overexpression or aberrant expression in other pathologic states.

A biological sample may be a cell sample, with an intervening culturing step being performed between the time the cell sample is collected from the subject and the immunoassay is carried out on the biological sample.

For immunohistological techniques, a tissue sample is collected from the subject, and the presence or absence of binding of an antibody of the invention is detected. The presence of binding of the antibody in an abnormal pattern or a pattern indicative of a tumor or cancer indicates the presence of a tumor or cancer in the subject from which the tissue sample is collected. The presence of the antigen in a metastatic tumor deposit can also be used to determine a likely source of the primary tumor. Any suitable immunohistology format may be used. The tissue sample may include patient biopsies, resections or cells for cytologic study. A similar technique to immunohistology is the use of similar techniques to detect and/or phenotype cells in body fluids or other suspensions as is used for flow cytometric examination.

For *in vivo* diagnostic purposes the antibody according to the invention is coupled to or provided with a suitable externally detectable label, such as e.g. a radiolabel as described above or a metal atom (e.g., technetium-99m), and

administered to a subject (*e.g.*, by intravenous or intraarterial injection), in an amount sufficient to produce an externally detectable signal, whereupon the possible localized accumulation of antibody in the body is determined, with a localized accumulation of the antibody (in a region other than that which would ordinarily be expected for normal subjects or subjects free of disease) indicating the present of a tumor in that subject.

4. Nucleic acid assay techniques.

Detection of mRNAs specific to EpoR isoforms 1, 2, 3, 4, and 5 may be carried out by any suitable technique, including but not limited to using reverse transcriptase-polymerase chain reaction (RT-PCR) amplification with isoform-specific primers and Southern blot analysis of the resulting RT-PCR amplicons. For example, PolyA⁺ RNA may be isolated by any technique known by those skilled in the art from patients patient cells and/or cancer cells, including but not limited to breast, colon, lung, ovary, and prostate cells or cancer cells. Methods for RT-PCR amplification of the isolated RNA are known in the art and may be carried out using EpoR isoform-specific primer pairs, preferably as described below.

Oligonucleotide probes (or primers) that specifically bind to a nucleic acid encoding an isoform as described above (including the opposite strands thereof), and pairs of probes (where at least one member of the pair is specific for a nucleic acid encoding one particular isoform), are also an aspect of the present invention. In general, such probes are from 8 or 10 nucleic acids in length up to 40 or 50 nucleic acids in length, or more. By "specifically bind" is meant that a probe binds to a nucleic acid (or complement thereof) that encodes one isoform as described herein, but does not bind to a nucleic acid (or complement thereof) that encodes another isoform as described herein. Probes may optionally be labeled with a detectable group such as a radioisotope, enzyme, or member of a binding pair in some assay formats. Where a pair of probes or primers is used for amplification, it will be appreciated that only one member of the pair need be isoform-specific, and that the other member of the pair may be one which will bind to nucleic acids encoding more than one of the isoforms described herein, so long as the primer pair specifically amplifies only nucleic acid encoding one of the isoforms described herein. Examples of such oligonucleotide probes, and pairs thereof, are as follows:

Primer pair specific for intron 6 insert (isoform 1)

28AS-: 5' TCA AGC GGC TGC TTC CTT CCA A 3' (SEQ ID NO: 14)

ER4-5: 5' GCA GGG AGC GTA CAG AGG GTG GAG 3' (SEQ ID NO: 15)

5 Primer pair specific for intron 7 insert (isoform 2)

33AS: 5' GAA GAA ATA GCA CCA ACC TGG AAG 3' (SEQ ID NO: 16)

31S: 5' CTG ACG CCT AGC GAC CTG GAC C 3' (SEQ ID NO: 17)

Primer pair specific for intron 7 unspliced (isoform 3)

10 31AS: 5' GCA GTT TGG CTG CAA GAA GCA 3' (SEQ ID NO: 18)

31S: 5' CTG ACG CCT AGC GAC CTG GAC C 3' (SEQ ID NO: 17)

Primer pair specific for intron 5 unspliced (isoform 4)

26S: 5' GGA GCC AGG GCG AAT CAC GG 3' (SEQ ID NO: 19)

15 32S: 5' GCC TTC AAA CTC GCT CTC TG 3' (SEQ ID NO: 20)

Primer pair specific for exon 6 skipped (isoform 5)

34AS 5' GCT TCA GAG CCC GCT AGG CGT 3' (SEQ ID NO: 21)

ER4-5 5' GCA GGG AGC GTA CAG AGG GTG GAG 3' (SEQ ID NO: 15)

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Note that, in the foregoing pairs, the primers of SEQ ID NO. 14, 16, 18, 19 and 21 are specific for the identified isoform, and the primers of SEQ ID NO. 15, 17 and 20 are not specific. In each pair, only one primer need be specific to provide an isoform-specific primer pair.

25 Blotting techniques are well known in the art. *See, e.g.,* Sambrook et al., *Molecular Cloning: a Laboratory Manual* 3rd Ed. (Cold Spring Harbor, NY,); Ausubel et al. *Current Protocols in Molecular Biology* (Green Publishing Associates, Inc. and John Wiley & Sons, Inc., New York). The nucleic acids resulting from RT-PCR amplification may be separated by gel electrophoresis and immobilized on a
30 suitable matrix, e.g. a filter of nitrocellulose. The presence of target sequences among the amplification products may be shown by incubation of the blotted amplicons with a probe (usually labeled) under conditions that promote denaturation and rehybridization. Because the probe is designed to base pair with target sequences, the probe will bind under renaturing conditions. Unbound probe is then removed, and

detection of target sequences may be accomplished via known techniques to detect the labeled probe.

The present invention and the various methods and compounds therein are explained in greater detail in the following non-limiting examples.

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Example 1

The Erythropoietin Receptor (EpoR) gene. The human EpoR gene has been cloned and sequenced as previously described (Noguchi et al. (1991) *Blood* **78**:2548-2556). The gene spans 8.6 kilobases, and comprises of 8 exons with 7 intervening
10 introns, the latter of which range in size from 81 bp to 2.1 kb. The organization of the EpoR gene is outlined in Figure 1. The full-length wild-type form of EpoR comprises of 508 amino acids (**SEQ ID NO: 2**) in three domains: extracellular, transmembrane (TM), and cytoplasmic. Exons 1-5 encode for the extracellular domain of EpoR, exon VI encodes for the transmembrane domain, while exons VII and VIII encode for the
15 cytoplasmic domain of the receptor. In the examination of EpoR expression in tumor vasculature, analysis by RT-PCR indicated a high level of EpoR mRNA expression in breast cancer cells, as well as squamous cell cancers of head-neck and uterine cervix was observed.

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Example 2

Novel Isoforms of EpoR mRNA Transcripts. The resulting RT-PCR amplification products derived from human cervix, breast, prostate, and ovarian cancer cell lines were sequenced and analyzed. The results of this study revealed five alternatively spliced EpoR mRNA transcripts that differ from the mature, full-length
25 wild-type EpoR mRNA. Using isoform-specific PCR primers, transcripts corresponding to each isoform were detected in breast, colon, lung, ovarian and prostate cancer. The organization of these five isoforms is outlined in Figure 1. The alternative forms of EpoR predicted to be coded for from these alternatively spliced mRNAs fall into two categories. Isoforms 1, 2, and 3 are described as truncated
30 (EpoR-T), and possess the extracellular and transmembrane domains of the wild-type receptor, while lacking portions of the cytoplasmic domain. Isoforms 4 and 5 are described as soluble (EpoR-S), and only possess the extracellular domain of the wild-type receptor intact. The changes in the putative C-terminal amino acid sequence encoded by these mRNAs are outlined in Figure 2.

Example 3

EpoR Isoform 1. The mRNA that codes for Isoform 1 contains an additional 114 nucleotides from intron 6 (nucleotides 5949-6062, **SEQ ID NO: 1**) spliced between exons 6 and 7. The resulting mRNA will code for an EpoR peptide 285 amino acids in length (**SEQ ID NO: 5**) with a severe truncation in the cytoplasmic region. At the C-terminal, 9 novel amino acids (M V R E G S R R R STOP) inserted at position 277 of the full-length EpoR peptide sequence.

Example 4

EpoR Isoform 2. The mRNA that codes for Isoform 2 is the result of an alternative splicing event between the 3' end of exon 7 and 5' end of exon 8, in which an additional 19 nucleotides (nucleotides 7498-7516, **SEQ ID NO: 1**) are added to the 5' end of exon 8. The mRNA from this splicing event codes for an EpoR peptide 317 amino acids in length (**SEQ ID NO: 7**) with a severe truncation in the cytoplasmic domain, in which 12 novel amino acids (V G A I S S A V A V P E STOP) are inserted at position 306 of the EpoR peptide sequence. As with Isoform 1, Isoform 2 also possesses a truncation of the cytoplasmic domain of the full-length peptide sequence of EpoR.

Example 5

EpoR Isoform 3. The translation of isoform 3 results from a processed EpoR mRNA in which sequences from intron 7 (nucleotides 7422-7516, **SEQ ID NO: 1**) are not spliced out of the final message. The resulting translation is a 328 amino acid peptide (**SEQ ID NO: 9**), with 23 novel amino acids introduced to the C-terminus (V G G L V V P S V P G L P C F L Q P N C R P L STOP) at position 306 of the EpoR peptide sequence. As with Isoforms 1 and 2, Isoform 3 possesses a truncation of the cytoplasmic domain of the full-length peptide sequence of EpoR. The sequence of the ORF of the mRNA message (**SEQ ID NO: 8**) and the peptide sequence of EpoR Isoform 3 (**SEQ ID NO: 9**) is identical to the translation predicted from an mRNA described previously (Nakamura et al. (1992) *Science* 257:1138-1141).

Example 6

EpoR Isoform 4. The processed EpoR mRNA that translates into Isoform 4 contains sequences from intron 5 (nucleotides 5061-5144, **SEQ ID NO: 1**) are not spliced out of the final message. The resulting translation is a 267 amino acid peptide
5 (**SEQ ID NO: 11**) with 21 novel amino acids (G E A P G G G V G G A R A N H G A S P P P STOP) introduced to the C-terminus at position 247 of the full-length EpoR peptide sequence. This isoform of EpoR possesses neither the transmembrane nor cytoplasmic domains of the full-length receptor. The translation that codes for Isoform 4 results in a soluble form of EpoR, containing the extracellular domain of
10 the receptor only.

Example 7

EpoR Isoform 5. Isoform 6 is a translation that results from the alternatively processed EpoR mRNA in which sequences from exon 6 are skipped, i.e. exons 5 and
15 7 are spliced together directly. The translation of this message results in a 248 amino acid peptide (**SEQ ID NO: 13**), in which 2 novel amino acids (G L STOP) are introduced at position 247 of the full-length peptide sequence of EpoR. As with Isoform 4, Isoform 5 of EpoR is a soluble form of the receptor that comprises of only the extracellular domain.

20 The foregoing is illustrative of the present invention, and is not to be construed as limiting thereof. The invention is defined by the following claims, with equivalents of the claims to be included therein.